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Conformation−Activity Relationships in Polyketide Natural Products: A New Perspective on the Rational Design of Epothilone Analogues

Richard E. Taylor,* Yue Chen, and Alicia Beatty†

Department of Chemistry and Biochemistry and the Walther Center for Excellence in Cancer Research, 251 Nieuwland Science Hall, University of Notre Dame, Notre Dame, Indiana 46556-5670

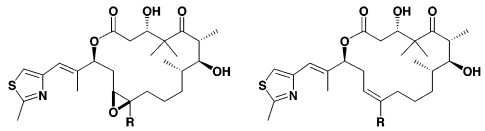
David C. Myles and Yiqing Zhou

Kosan Biosciences Inc., 3832 Bay Center Place, Hayward, California 94545

Received August 19, 2002; E-mail: taylor.61@nd.edu

The promising activities of the epothilones in in vitro and in vivo cancer models have attracted significant attention from the synthetic, biological, and medicinal communities.[1] Several epothilones are currently in clinical trials against a range of cancer types. These efforts have yielded hundreds of analogues in this exciting class and have identified the critical structural features necessary for biological activity. Despite these efforts, the role that the conformation of the epothilone macrocycle plays in displaying the pendant functional groups has gone under-recognized.

It has been a goal of our program to design analogues of complex polyketide macrolides to probe the effect of conformation on biological activity. In this contribution, we report epothilone analogues in which conformational families have been stabilized through rational substitution. This approach provides insights into the biologically active conformation of the epothilone class of natural products.



1a R = H epothilone A
2a R = Me epothilone B

1b R = H epothilone C
2b R = Me epothilone D

We have used a combination of computational methods and high-field NMR experiments to study the conformational properties of the epothilones.[2] Our previous report concentrated on the C1−C8 polypropionate region and concluded that the epothilones populate two major conformational families in solution, with the distribution of conformers being controlled by *syn*-pentane interactions. We also showed that the major contributor was related to the conformation observed in the solid state.[3]

Previously reported structure−activity relationships have shown that neither the presence nor the stereochemistry of the epoxide is essential to biological activity of these compounds.[1c] Conformational analysis of this region of the macrolide suggested that accessible conformations could be clustered into two families shown as I and II (R = H, Figure 1). Several groups have reported models of the epothilone pharmacophore and its relationship to other classes of microtubule-stabilizing natural products based on computational methods and reported structure−activity relationships.[4] In fact, two of these studies have proposed that the bioactive conformation of the epothilone epoxide region is similar to conformer II.[4a,b,5] In an effort to distinguish conformers I and II and to determine which is more closely related to the biologically active conformation of the epothilones, we have considered simple C14-methyl substituted

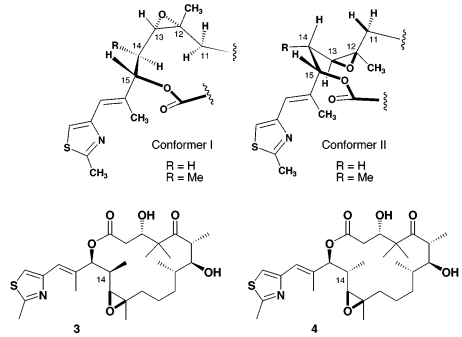
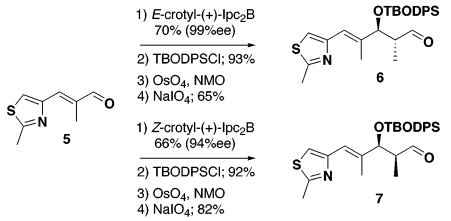


Figure 1. Conformations of the C10−C15 epoxide region.

compounds **3** and **4.** We rationalized that **3** and **4** would preferentially exist in conformers I and II (R = Me), respectively. In addition, the presence of the C14-methyl substituent would destabilize the alternative conformer. These preferences were supported by computer modeling techniques similar to our previous analysis of the natural products.[2]

The target compounds were prepared by a route based on our previously reported synthesis of epothilone B.[6] Thiazole aldehyde **5**, an intermediate in several synthetic routes to these compounds, is the point of divergence for the synthesis of **3** and **4**, Scheme 1.

Scheme 1



Brown asymmetric crotylboration[7] efficiently controlled the enantio- and diastereoselectivity of the C14, C15 stereogenic centers. Protection of the secondary hydroxyl as a *tert*-butoxydiphenylsilyl ether followed by oxidative cleavage provided aldehydes **6** and **7**.

The conversion of each of aldehydes **6** and **7** to epo D analogues **8** and **9** proceeded efficiently though identical synthetic sequences. Full details of their syntheses are included in the Supporting Information. (*S*)-14-methyl epothilone D **8** underwent a highly selective epoxidation with mCPBA to provide (*R*)-14-methyl epothilone B **3** in 55% yield, Scheme 2.

Epoxidation of (*R*)-14-methyl epothilone D **9** also proceeded in a highly selective fashion, Scheme 3. We believe that **9** will exist primarily in conformer II due to the conformational constraints imposed by the C14-methyl substituent (vide infra). Therefore, it

†To whom questions regarding the X-ray diffraction studies should be addressed.

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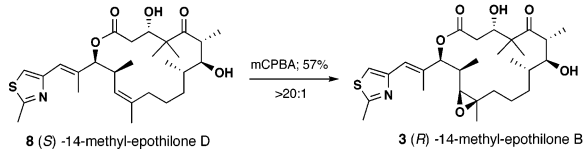
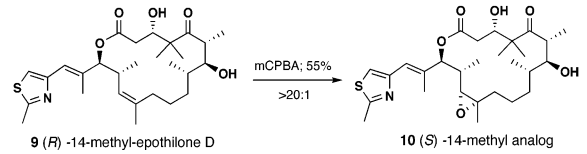
Scheme 2



8 (*S*) -14-methyl-epothilone D — **3** (*R*) -14-methyl-epothilone B

Scheme 3



9 (*R*) -14-methyl-epothilone D — **10** (*S*) -14-methyl analog

is expected that the stereochemistry of the epoxide is epimeric to epothilone B and analogues **3** and **4**. We formulate this epoxide as **10**.

X-ray diffraction studies of single crystals of **3** and **8**·H_2O (not shown) showed that the conformation of each in the solid state was quite similar to that reported for epothilone B and thus conformer I in the epoxide region,[8] Figure 2. Proton NMR coupling

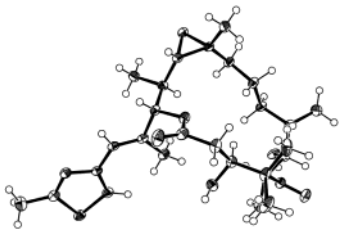


Figure 2. Solid-state structure of **3**.

constants (J_{14-15} = 9.9, 10.5 Hz respectively) also supported this preference in solution. In contrast, proton NMR coupling constants of analogues **9** and **10** had the expected values for conformer II (J_{14-15} = 3.3, <2.0 Hz respectively). Computational models suggested a H−C_{14}−C_{15}−H dihedral angle of 175° (J_{calc} = 10.9 Hz) in **3** (conformer I) and 62° (J_{calc} = 3.1 Hz) in **4** (conformer II). Additional NMR evidence for these conformational differences were observed in ROESY experiments with **8**, **3**, **9**, and **10**.[9] An NOE enhancement between H_{13} and the C_{16}−CH_3 was only observed in analogues **9** and **10** but not in **8** and **3**. These data not only support the preference for conformer II but also the proposed configuration of epoxide **10**.

The biological activities of epothilone analogues **3**, **8**, **9**, and **10** were evaluated against a panel of human tumor cell lines (Table 1). From this study, it is clear that the stereochemistry of the newly

Table 1. Cytotoxicity of Epothilone Analogues (IC_{50} nM)

compound	MCF-7	NCI/ADR	H460	SF
2a	1.5	3.6	1.7	0.7
2b	5	26	20	7
3	3	23	3	3
8	35	238	42	42
9	>1000	>1000	>1000	>1000
10	>1000	>1000	>1000	>1000

introduced methyl group at C14 has a significant impact on the biological activity of the epothilone analogues. Compounds **3** and **8** (conformer I preference) maintain significant cytotoxicity. In marked contrast, analogues **9** and **10** (conformer II preference) showed no measurable cytotoxicity.

The conformation−activity relationships presented herein strongly support the importance of conformer I as the bioactive conformation of the 12,13-epoxide (olefin) region of the epothilones. The approach presented here offers a new perspective on rational design of modified biologically active polyketide macrolides. The recent advances in genetic engineering of polyketide synthases (PKS)[10] may provide an alternative synthetic route to these and related conformationally restricted analogues through manipulation of the epothilone PKS gene cluster.[11] Efforts along these lines are currently being pursued. Additional conformational analogues of epothilone have been prepared in our laboratory and the results will be reported in due course.

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Supporting Information Available: Full experimental and characterization data for the preparation of **3**, **8**, **9**, and **10** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org

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(8) The authors have deposited the crystallographic data for **3** and **8**·H_2O with the Cambridge Crystallographic Data Center (CCDC 191723 and 191724). The data can be obtained, on request, from the Director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, U.K or www.ccdc.cam.ac.uk/conts/retrieving.html.
(9) A strong NOE enhancement was observed between the H_{14} and one of the C_{11} protons in both **8** and **9**. In addition a strong NOE enhancement was observed between H_{15} and H_{13} in epoxide **3** but not in epoxide **10**. An NOE enhancement between H_{14} and H_{15} was only observed in analogues **9** and **10** but not in **8** and **3**. The observed coupling constants between H_{13} and H_{14}, in all four analogues, were similar (J_{13-14} = 8.1−9.9 Hz). Attempts to obtain single crystals of **9** and **10** were unsuccessful.
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